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Etiology and Pathophysiology/Obesity Comorbidity

Role of gut microbiota in chronic low-grade inflammation as potential driver for atherosclerotic cardiovascular disease: a systematic review of human studies

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Summary

A hallmark of obesity is chronic low-grade inflammation, which plays a major role in the process of atherosclerotic cardiovascular disease (ACVD). Gut microbiota is one of the factors influencing systemic immune responses, and profound changes have been found in its composition and metabolic function in individuals with obesity. This systematic review assesses the association between the gut microbiota and markers of low-grade inflammation in humans. We identified 14 studies which were mostly observational and relatively small ($n = 10$ to 471). The way in which the microbiome is analysed differed extensively between these studies. Lower gut microbial diversity was associated with higher white blood cell counts and high sensitivity C-reactive protein (hsCRP) levels. The abundance of *Bifidobacterium*, *Faecalibacterium*, *Ruminococcus* and *Prevotella* were inversely related to different markers of low-grade inflammation such as hsCRP and interleukin (IL)-6. In addition, this review speculates on possible mechanisms through which the gut microbiota can affect low-grade inflammation and thereby ACVD. We discuss the associations between the microbiome and the inflammasome, the innate immune system, bile acids, gut permeability, the endocannabinoid system and TMAO. These data reinforce the importance of human research into the gut microbiota as potential diagnostic and therapeutic strategy to prevent ACVD.

Keywords: Atherosclerotic cardiovascular disease, gut microbiome, inflammation, obesity.

Abbreviations: ACVD, atherosclerotic cardiovascular disease; hsCRP, high sensitivity C-reactive protein; IL, interleukin; TNF- α , tumour necrosis factor alpha; PRRs, pattern recognition receptors; LPS, lipopolysaccharide; LGC, low gene count; *L. reuteri*, *Lactobacillus reuteri*; qPCR, quantitative polymerase chain reaction; *F. prausnitzii*, *Faecalibacterium prausnitzii*; *E. coli*, *Escherichia coli*; *C. nexile*, *Clostridium nexile*; *B. fragilis*, *Bacteroides fragilis*; *Staph.aureus*, *Staphylococcus aureus*; IFN- γ , interferon gamma; *A. muciniphila*, *Akkermansia muciniphila*; *R. gnavus*, *Ruminococcus gnavus*; *P. nigrescens*, *Prevotella nigrescens*; BMI, body mass index; PAMPs, pathogen-associated molecular patterns; TLR, toll-like receptor; NOD, nucleotide-binding oligomerization domain; HFD, high fat diet; SCFAs, short chain fatty acids; Treg, regulatory T cells; GPR, G protein coupled receptors; GLP-2, glucagon-like peptide 2; eCB system, endocannabinoid system; NLRP6, NOD-like receptor family pyrin domain containing 6; TGF- β , transforming growth factor beta; TMAO, trimethylamine-N-oxide.

Introduction

The worldwide pandemic of obesity has led to an increased prevalence of cardiovascular risk factors (diabetes mellitus, hypertension and dyslipoproteinemia) and atherosclerotic cardiovascular disease (ACVD). This complex of cardiovascular risk factors is labelled 'the metabolic syndrome', which is associated with a twofold higher risk for ACVD (1). Losing weight is notoriously difficult and most often only temporary. Interestingly however, not all individuals with obesity develop the metabolic syndrome and resulting ACVD. Approximately 20–30% of the individuals with obesity are metabolically healthy, and their risk for ACVD is substantially lower than in individuals with metabolically unhealthy obesity (2,3). A better understanding of metabolically healthy obesity might offer novel treatment strategies to prevent ACVD in patients that are overweight (4). Individuals with obesity that develop ACVD are often characterized by a chronic low-grade inflammatory status, described as 'meta-inflammation' (5,6). It has been hypothesized that differences in inflammatory status partly explain the individual differences in cardiovascular risk.

Since the discovery that tumour necrosis factor (TNF)- α is overexpressed in the adipose tissue of mice with obesity, numerous studies have focused on the role of adipose tissue in the development of inflammation (7). The current hypothesis states that fat mass expands due to a positive energy balance, which leads to a pro-inflammatory reaction of both adipocytes and surrounding immune cells. While initially considered to be of little relevance for metabolic dysregulation and cardiovascular risk in obesity-related disease, the modest increases in circulating inflammatory mediators have in recent years been found to be strongly associated with the development of non-alcoholic fatty liver disease, type 2 diabetes and atherosclerosis (8,9). These pro-inflammatory pathways are partly mediated via pattern recognition receptors (PRRs). The beneficial effects of blocking inflammatory pathways on ACVD were recently underscored by the results of the CANTOS trial. Canakinumab, a human monoclonal antibody that inhibits the pro-inflammatory cytokine interleukin (IL)-1 β reduced cardiovascular death, non-fatal myocardial infarction and non-fatal stroke (10). Despite this progress, the initial triggers of inflammation in patients with metabolic dysregulation remain obscure.

Several studies suggest that the obesity-related systemic inflammation is at least partly driven by an altered gut microbial composition and function (11). The human gut microbiota consists of approximately 10^{13} – 10^{14} microbes, including bacteria, viruses, fungi and protozoa. Besides their function in intestinal epithelial homeostasis, development of the immune system, protection against pathogens and energy homeostasis (12–15), the gut

microbiota also plays a role in pathophysiological mechanisms associated with different diseases (16).

Profound changes have been observed in the composition and metabolic function of gut microbiota in individuals with obesity. Obesity was first reported to be associated with a change of gut microbiota by Ley *et al.* in 2005; characterized by obesity, ob/ob mice appeared to have less phylum Bacteroidetes and more Firmicutes compared with lean phenotypes (17). A further major observation came from Turnbaugh *et al.* who reported that the core gut microbiome of individuals affected by obesity has an increased capacity for energy harvesting (18).

To explore whether the gut microbiota contributes to chronic inflammation as driver for ACVD in humans, we conducted a systematic review of studies investigating the role of the gut microbiome in chronic low-grade inflammation. In this review, we also discuss possible mechanisms by which the gut microbiota can influence chronic low-grade inflammation and thereby contribute to the development and progression of ACVD.

Methods

Identification and selection of articles

A systematic review of peer-reviewed studies examining the role of the gut microbiome on chronic low-grade inflammatory markers in human populations was undertaken. A protocol was developed *a priori*, outlining the review aim and procedure. The literature search was conducted using Medline (Pubmed), CINAHL and the Cochrane Library from inception until November 2017. Key MeSH subject terms and keywords pertaining to the gut microbiome and inflammation in correlation to obesity or ACVD were included. The following search string was employed: (('Gastrointestinal Microbiome') OR ('Gut microbiome')) AND (('Inflammation') OR ('Inflammation Mediators')) OR ('Host-Pathogen Interactions') OR ('Immune System') OR ('Immunity') OR ('Adaptive Immunity')) AND (('Obesity') OR ('Overweight') OR ('Cardiovascular Diseases') OR ('Coronary Disease') OR ('Atherosclerosis')) Filters: *Humans*.

Two reviewers (I.v.d.M. and J.R.) independently evaluated eligibility of studies based on the title and abstract using the following inclusion criteria: (i) participants older than 18 years of age; (ii) investigation of the gut microbiome; (iii) investigation of a quantitative measure of inflammation, e.g. inflammatory cell count, hsCRP, cytokines or a potential trigger for inflammation like circulating lipopolysaccharide (LPS); and (iv) full text availability in the English language. The presence of infectious or inflammatory diseases (i.e. inflammatory bowel disease, HIV and rheumatoid arthritis) in the study population was the only exclusion criterion.

Afterwards, full-text articles were assessed independently by two reviewers (I.v.d.M. and J.R.). We also checked for cited articles in original research articles and reviews that addressed the mechanistic links between the gut microbiome and low-grade inflammation.

Study quality assessment

The included observational studies were assessed on their quality using an adapted scale from the Newcastle–Ottawa scale for quality assessment of cohort and case–control studies (NOS scale). This scale is a modified version of the NOS scale, as also used by several other studies (19,20). For the included non-randomized intervention studies, we used the conventional NOS scale. A study with a NOS score of 7 or more can be considered to be a study of ‘good’

quality (21). For the randomized intervention studies, we used the Cochrane risk of bias 2.0 (22).

Results

Data extraction and quality assessment

The search process identified 629 articles for potential inclusion. In total, 260 manuscript contained original research data. After reviewing the titles and abstracts, 61 studies met the initial inclusion criteria (Fig. 1). Following the initial selection, another 51 studies were excluded, mainly because they were not original studies or the study did not investigate the direct relation between the gut microbiome and inflammatory markers at baseline, leading to inclusion of 10 studies. We also checked the reference list of the 61 non-

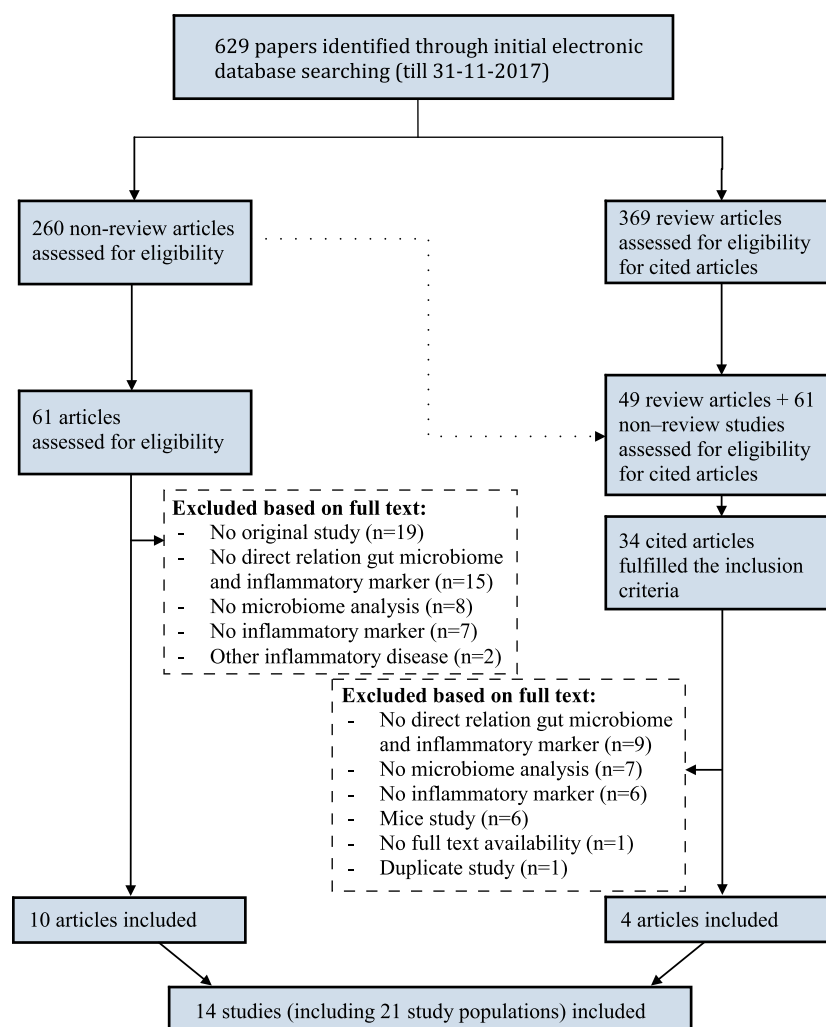


Figure 1 PRISMA flow diagram with schematic presentation of the study assessment and exclusion stages. [Colour figure can be viewed at wileyonlinelibrary.com]

review studies that initially met the inclusion criteria. Additionally, we checked 369 reviews, of which 49 were of interest and of which we checked the reference list. In total, 34 original manuscripts met the inclusion criteria. However, the majority of these studies were excluded, because they did not report on microbiome analysis or used a surrogate marker to assess the gut microbiome. An additional five articles were identified via these reference lists for cited articles. We identified one study that presented a data set that was published more than once (trial registered as ISRCTN88720134). For this data set, we selected the study reporting on the largest cohort (23). Two other studies used the same data set; however, they performed different

analyses (24,25). Therefore, both studies were included. Finally, this resulted in 14 unique manuscripts. The 14 articles included 21 populations with a total of 1,418 individuals (see Tables 1 and S1 for detailed information of each study). For the intervention studies, we only included the data at baseline in order to overcome the effect of the intervention on the relation between the gut microbiome and inflammation.

Eight out of the 11 observational and non-randomized studies were considered to be studies of good quality based on the NOS score (Tables S2 and S3). The methodological quality of the randomized intervention studies ranged from low risk to high risk for bias (three studies; Table S4).

Table 1 Detailed information of all studies included in this systematic review

Study – year	Ref	Country	Population	Mean age	Mean BMI	Study design	Statistical analyses	Method microbiome analysis
Schirmer <i>et al.</i> – 2016	27	The Netherlands	471 healthy subjects	29 ± 14	23 ± 3	Observational	Spearman correlation with Benjamini–Hochberg correction	Quantitative metagenomics
Dao MC <i>et al.</i> – 2016	24	France	49 subjects with overweight	42 ± 12	33 ± 1	Dietary intervention	Kruskal–Wallis test with Bonferroni correction	Quantitative metagenomics
Radilla-Vázquez RB <i>et al.</i> – 2016	35	Mexico	32 subjects with obesity and 32 control subjects	21 ± 2 and 21 ± 2	21 and 35	Observational	Kruskal–Wallis test	qPCR
Rajkumar H <i>et al.</i> – 2014	33	India	60 subjects with overweight	49	29	Randomized controlled trial with probiotic	ANOVA	Culturing
Le Chatelier E <i>et al.</i> – 2013	26	Denmark	169 subjects with obesity and 123 control subjects	57	30	Observational	Linear model adjusting for age and sex; Benjamini–Hochberg method	Quantitative metagenomics
Cotillard A <i>et al.</i> – 2013	25	France	38 subjects with obesity and 11 subjects with overweight	44 ± 2 and 46 ± 3	49 ± 1 and 28 ± 1	Dietary intervention	Mann–Whitney test	Quantitative metagenomics
Clemente-Postigo M <i>et al.</i> – 2013	23	Spain	10 subjects	48 ± 2	28 ± 3	Randomized cross-over trial with alcohol intervention	Pearson's correlation test	qPCR
Martínez I <i>et al.</i> – 2013	31	USA	28 healthy subjects	26 ± 6	25 ± 5	Randomized cross-over trial with dietary intervention	Pearson's correlation test	16S rRNA gene analysis
Claesson MJ <i>et al.</i> – 2012	30	Ireland	165 older and 13 young subjects	78 ± 8 and 36 ± 6	27 ± 5 and?	Observational	Linear quantile regression	16S rRNA gene analysis
Brignardello J <i>et al.</i> – 2010	52	Chile	6 subjects with obesity and 6 control subjects	34 ± 12 and 30 ± 8	36 ± 5 and 24 ± 2	Observational	Spearman rank test	G + C peak content analysis
Mikelsaar M <i>et al.</i> – 2010	28	Estonia	38 older subjects	72 ± 5	27 ± 4	observational	Linear multiple regression analysis adjusted for age, sex and BMI	qPCR
Furet JP <i>et al.</i> – 2010	32	France	30 subjects with obesity and 13 control subjects	44 ± 2 and 36 ± 3	48 ± 2 and 22 ± 0	Bariatric surgery intervention	Principal component analysis combined with Spearman analysis	qPCR
Biagi E <i>et al.</i> – 2010	34	Italy	84 subjects	25–104		Observational	Pearson's correlation test	Microarray analysis, qPCR
Tiihonen K <i>et al.</i> – 2010	29	Finland	20 subjects with obesity and 20 control subjects	33 ± 2 and 23 ± 2	33 ± 2 and 23 ± 2	Observational	Pearson's correlation test	qPCR

Circulating immune cell counts

Three studies investigated circulating immune cell counts as marker of inflammation. The details of these studies and the results are summarized in Table 2a. A low gene count (LGC) of the gut microbiome correlated with a higher white blood cell count in a large Danish population-based study employing metagenomics analysis (26). Another large Dutch cohort study that included mainly healthy younger individuals did not find a relationship between the gut microbiome composition and white blood cell count (27). In a small study in elderly, a higher white blood cell count correlated with the presence of the species *Lactobacillus reuteri* (*L. reuteri*) as analysed by quantitative polymerase chain reaction (qPCR) (28).

C-reactive protein

The majority of studies investigating the relation between the gut microbiome and low-grade inflammation used (hs) CRP as marker. In Table 2b, we provide an overview of the details and results of these studies. An LGC phenotype was associated with increased hsCRP levels in the aforementioned Danish population-based study (26) and revealed a tendency towards an increased hsCRP in another smaller diet intervention study (25). The total bacterial cell counts related inversely with hsCRP in another study (29). Six studies examined the relationship between the phylum or species abundance with (hs) CRP levels. The largest study included 165 older individuals and specifically measured 16S rRNA by massive parallel sequencing finding that lower levels of the *Oscillibacter* co-abundance group and higher levels of the *Bacteroides* co-abundance group coincided with increased levels of CRP (30). Two other small studies reported that lower levels of the genera *Faecalibacterium* and *Ruminococcus*, and *Faecalibacterium prausnitzii* (*F. prausnitzii*) species correlated with increased hsCRP levels (31,32). These studies were fairly similar in both samples sizes and methods of microbiome analysis. The first study was a randomized cross-over trial with 4-week diet intervention that included 28 young adults and used 16S rRNA gene sequencing to reconstruct microbiome taxonomic composition (31). The second study was an intervention study with bariatric surgery in 30 individuals with obesity. They analysed the gut microbiota by 16S rRNA gene sequencing combined with qPCR analysis (32). For the current review, we only considered the correlation results at baseline from both studies. Next to that, the study by Rajkumar *et al.* who found that lower levels of the genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus* and higher levels of the species *Escherichia coli* (*E. coli*) correlated with increased (hs) CRP in 60 healthy adults affected by overweight (33), used bacterial culturing methods to identify bacteria. Plates

were incubated in triplicate using selective media for enumeration of total aerobes, anaerobes, coliforms, *E. coli*, *Bifidobacterium*, *Lactobacillus* and *Streptococcus thermophilus*.

Cytokines

Six studies investigated relationships between the abundance of gut microbiome species and pro-inflammatory cytokines. The details and results of these studies are summarized in Table 2c. The total bacterial cell count was positively related to circulating TNF- α levels in a small Finnish cohort study using qPCR as microbiota analysis method (29). The largest study on the relationship between specific genera, species and cytokines was mentioned before and included 165 older individuals (30). Lower levels of the *Ruminococcus* and *Prevotella* co-abundance group, as well as higher levels of the *Oscillibacter* co-abundance group, coincided with higher IL-6 levels. Two small studies mentioned in the hsCRP section also reported a negative correlation between the family *Ruminococcaceae* (31) and species *F. prausnitzii* (32) with IL-6 levels. The Italian study that included four different age groups, ranging between 25 and 104 years of age (35), showed a negative correlation between IL-6 and species *Eubacterium hallii*, *Eubacterium ventriosum*, *Eubacterium rectale*, *Clostridium nexile* (*C. nexile*) and species in the *Clostridium cluster XIVa*. A positive correlation with circulating IL-6 was observed for the genera *Haemophilus*, *Pseudomonas*, *Serratia*, *Yersinia*, *Vibrio* and *Bacillus*, and species *Eggerthella lenta*, *Eubacterium cylindroides*, *E. coli* and *Klebsiella pneumoniae*. Increased levels of the genera *Lemniscella*, *Proteus* and *Bacillus*, and species *Alcaligenes faecalis*, *Eggerthella lenta* and *Eubacterium cylindroides* correlated with higher levels of IL-8, while decreased levels of the species *Eubacterium hallii*, *Eubacterium ventriosum*, *Eubacterium rectale*, *C. nexile* and species within the *Clostridium cluster XIVa* related to higher IL-8 levels; this study investigated the gut microbiome by microarray and qPCR analysis (35).

Among all microbiome–IL association studies, Schirmer *et al.* included the largest number of individuals, $n = 471$, all healthy and mainly young. They investigated cytokine responses *ex vivo* in peripheral blood mononuclear cells (PBMCs) and whole blood stimulated with five different microbial pathogens in relation to microbial taxonomy and functionality, as analysed by metagenomics (27). For the purpose of this review, we have focused on the bacterial taxa and bacteria-derived stimulations: *E. coli*-derived LPS, *Bacteroides* (*B.*) *fragilis* representing gram-negative bacteria and *Staphylococcus* (*Staph.*) *aureus* representing gram-positive bacteria. For these stimulations, three monocyte-derived cytokines (IL-1 β , IL-6 and TNF- α) and three lymphocyte-derived cytokines (interferon [IFN]- γ , IL-17 and IL-22) were measured. No association was found

Table 2 Summary of findings of included studies in this systematic review per marker of inflammation

Table 2a Gut microbiome measurements in relation to white blood cell counts

Study	Summary of finding
Le Chatelier <i>et al.</i> (26)	The phenotype of low gene count people was associated with a more marked inflammatory phenotype with higher white blood cell counts, model adjusted for age and sex ($q = 0.014$; $P = 0.002$).
Schirmer <i>et al.</i> (27)	No effect of microbial composition on the most important immune cell population (T/B lymphocytes, monocytes, neutrophils, NK cells) was detected.
Mikelsaar M <i>et al.</i> (28)	The higher white blood cells count was positively related ($r = 0.434$, $P = 0.007$) to the presence of <i>L. reuteri</i> , also after adjustment for age, sex and BMI (adj. $R^2 = 0.193$, $P = 0.027$).

Table 2b Gut microbiome measurements in relation to (hs)CRP

Study	Summary of finding
Le Chatelier E <i>et al.</i> (26)	The phenotype of low gene count people was associated with a more marked inflammatory phenotype with increased hsCRP ($q = 0.012$; $P < 0.001$).
Cotillard A <i>et al.</i> (25)	The low gene count group had a trend towards higher inflammation (hsCRP).
Tiihonen K <i>et al.</i> (29)	An inverse correlation between the total faecal microbial counts and serum hsCRP ($r = -0.51$, $P = 0.04$) was found.
Dao MC <i>et al.</i> (24)	No difference in hsCRP between high and low <i>Akkermansia</i> group
Claesson MJ <i>et al.</i> (30)	A reduction in the <i>Oscillibacter</i> co-abundance group and increase in the <i>Bacteroides</i> co-abundance group, coincided with increased levels of CRP.
Martínez I <i>et al.</i> (31)	Negative correlation between the family <i>Ruminococcaceae</i> ($r = -0.59$, $P = 0.0024$) with hsCRP were revealed. Within this family, the genera <i>Faecalibacterium</i> and <i>Ruminococcus</i> displayed negative correlations with hsCRP ($r = -0.48$, $P < 0.05$ and $r = -0.60$, $P < 0.01$, respectively).
Furet JP <i>et al.</i> (32)	The strongest associations were found for the amount of the species <i>F. prausnitzii</i> , which was negatively correlated with serum concentrations of inflammatory circulating markers, hsCRP (Rs -0.54 , $P < 0.01$)
Rajkumar H <i>et al.</i> (33)	Subjects with more than 3 mg L^{-1} hsCRP has significantly lower <i>Lactobacilli</i> , <i>Bifidobacteria</i> , and <i>Streptococcus</i> and higher <i>E. coli</i> when compared with those who had less than 3 mg L^{-1} .
Brignardello J <i>et al.</i> (34)	The G + C peak values correlated negatively with the higher CRP levels ($r = -0.68$; $P < 0.02$).

Table 2c Gut microbiome measurements in relation to cytokines

Study	Summary of finding
Schirmer <i>et al.</i> (27)	At genus level: A negative relation was present between <i>Roseburia</i> and IL-6 levels (after <i>B. fragilis</i> stimulation). A negative relation was present between <i>Faecalibacterium</i> , <i>Atopobium</i> and IL-17 levels (after <i>Staph. aureus</i> stimulation). A positive relation was present between <i>Escherichia</i> , <i>Anaerotruncus</i> , <i>Coprobacillus</i> , <i>Clostridium</i> and <i>Anaerostipes</i> and IL-17 levels (after <i>Staph. aureus</i> stimulation). A negative relation was present between <i>Oscillibacter</i> (after LPS stimulation), <i>Barnesiella</i> (after LPS and <i>B. fragilis</i> stimulation), <i>Leuconostoc</i> (after <i>B. fragilis</i> stimulation) and IFN- γ levels. A positive relation was present between <i>Megasphaera</i> (after <i>B. fragilis</i> stimulation) and IFN- γ levels. A negative relation was present between <i>Bilophila</i> , <i>Odoribacter</i> and TNF- α levels (after LPS stimulation). A positive relation was present between <i>Methanosphaera</i> and TNF- α levels (after LPS stimulation). At species level: A negative relation was present between <i>Lachnospiraceae bacterium 5 1 65FAA</i> and IL-6 levels (after <i>Staph. aureus</i> stimulation). A negative relation was present between <i>Parabacteroides johnsonii</i> and IL-17 levels (after <i>Staph. aureus</i> stimulation). A positive relation was present between <i>Escherichia spp.</i> , <i>B. intestinalis</i> , <i>Anaerotruncus spp.</i> and IL-17 levels (after <i>Staph. aureus</i> stimulation). A negative relation was present between <i>B. eggerthii</i> , <i>Coprococcus comes</i> and IL-22 levels (after <i>Staph. aureus</i> stimulation). A positive relation was present between <i>B. cellulosilyticus</i> and <i>R. gnavus</i> (both after <i>Staph. aureus</i> stimulation), <i>Megasphaera sp.</i> , <i>Eubacterium limosum</i> , <i>B. nordii</i> (all after <i>B. fragilis</i> stimulation) and IFN- γ levels. A negative relation was present between <i>Lachnospiraceae bacterium 9 1 57FAA</i> and IFN- γ levels (after <i>Staph. aureus</i> stimulation). A negative relation was present between <i>Clostridium leptum</i> , <i>Alistipes finegoldii</i> , <i>Bilophila spp.</i> , <i>Bilophila wadsworthia</i> , <i>Alistipes onderdonkii</i> , <i>Enterococcus faecium</i> , <i>Collinsella intestinalis</i> , <i>Odoribacter splanchnicus</i> and TNF- α levels (after LPS stimulation). A positive relation was present between <i>Acidaminococcus intestini</i> , <i>Methanosphaera stadmanae</i> , <i>L. acidophilus</i> and TNF- α (after LPS stimulation)
Claesson MJ <i>et al.</i> (30)	A reduction in abundance of <i>Ruminococcus</i> and <i>Prevotella</i> and increased abundance of <i>Oscillibacter</i> co-abundance groups was accompanied by an increase in IL-6 levels.
Martínez I <i>et al.</i> (31)	A negative correlation between the family <i>Ruminococcaceae</i> and IL-6 was revealed ($P < 0.05$).
Biagi E <i>et al.</i> (35)	IL-8 correlated positively with <i>Alcaligenes faecalis et rel</i> , <i>Leminorella</i> , and <i>Proteus et rel</i> , <i>Bacillus</i> , <i>Egghertella lenta et rel</i> and <i>Eubacterium cylindroides et rel</i> . IL-6 correlated positively with <i>E. coli et rel</i> , <i>Haemophilus</i> , <i>Klebsiella pneumoniae et rel</i> , <i>Pseudomonas</i> , <i>Serratia</i> , <i>Yersinia et rel</i> and <i>Vibrio</i> , <i>Bacillus</i> , <i>Egghertella lenta et rel</i> and <i>Eubacterium cylindroides et rel</i> . <i>Eubacterium halli</i> , <i>Eubacterium ventriosum</i> , <i>Eubacterium rectale</i> , <i>C. nexile</i> and <i>Clostridium cluster XIVa</i> were inversely correlated with IL-6 and IL-8.
Tiihonen K <i>et al.</i> (29)	Serum TNF- α was associated with the total number of bacteria ($r = 0.42$, $P = 0.006$).

(Continues)

Table 2 (Continued)

Table 2a Gut microbiome measurements in relation to white blood cell counts

Furet JP *et al.* (32) The strongest associations were found for the amount of *F. prausnitzii*, which was negatively correlated with serum concentrations of inflammatory circulating markers, IL-6 (Rs -0.65 , $P < 0.001$)

Table 2d Gut microbiome measurements in relation to LPS

Study	Summary of finding
Dao MC <i>et al.</i> (24)	No difference in LPS between high and low <i>Akkermansia</i> group
Radilla-Vázquez RB <i>et al.</i> (36)	A low number of <i>E. coli</i> comes along with a greater risk of having high LPS levels (OR = 4.378, $P = 0.005$). However, a positive correlation between LPS and <i>E. coli</i> was only found in the second tertile (LPS = 1–1.3 EU/mL) presenting fewer bacteria compared with the first (LPS < 1 EU/mL) and third (LPS > 1.3 EU/mL) tertiles ($P = 0.036$).
Clemente-Postigo M <i>et al.</i> (23)	LPS concentrations correlated negatively with <i>P. intermedia</i> ($r = -0.336$, $P = 0.037$) and with the genus <i>Bifidobacterium</i> ($r = -0.411$; $P = 0.009$).

between bacteria-derived stimulations and IL-1 β . On the other hand, IL-6 production after *Staph. aureus* stimulation was negatively related to the species *Lachnospiraceae bacterium* 5 1 65FAA. After stimulating PBMCs with LPS, TNF- α production was negatively related with *Alistipes spp*, *Clostridium spp* and *Bilophila spp* among others. For IL-17, 5 positive associations with genera were identified, including genus *Clostridium*, as well as two negative correlations, with *Faecalibacterium* and *Atopobium*. A differential IFN- γ response was observed for all bacterial stimulations.

Circulating lipopolysaccharide

Three studies investigated the relationship between the gut microbiome and circulating LPS levels. The details of these studies and the results are summarized in Table 2d. In a cohort study of 49 individuals with overweight, no relationship was observed for *Akkermansia muciniphila* (*A. muciniphila*) with LPS levels (24). A very small Spanish study of 10 individuals found LPS concentrations to be negatively correlated with the genera *Prevotella* and *Bifidobacterium* (23). Another Mexican study of 60 individuals, analysed the gut microbiota by qPCR and found that lower levels of the species *E. coli* correlated with lower levels of LPS; however, a positive correlation between LPS and *E. coli* was only found in the second tertile (LPS = 1–1.3 EU/mL) presenting fewer *E. coli* compared with the first (LPS < 1 EU/mL) and third (LPS > 1.3 EU/mL) tertiles (36).

Discussion

Given the increased prevalence of obesity worldwide, the incidence of ACVD is concomitantly on the rise. Because ACVD events can only be partly explained by traditional cardiovascular risk factors, the gut microbiota is a new area to explore for new diagnostic and therapeutic tools in ACVD. It has already been shown that the gut microbiome contributes to a substantial proportion of the variation in blood lipids (37). Because chronic low-grade inflammation is one of the hallmarks of obesity, this provides another route for the gut microbiota to influence the atherosclerotic

process. To our knowledge, this is the first systematic review of human studies investigating relationships between the gut microbiota and markers of chronic low-grade inflammation. Several associations between the gut microbiota and different markers of chronic low-grade inflammation were identified. A low gut microbial diversity has previously been reported in individuals with obesity compared with lean individuals. However, not just the diversity in gut microbiota, but also the presence of specific species has been shown to correlate with chronic low-grade inflammatory markers.

Association of gut microbiome with inflammation

A lower alpha diversity and gene count of the gut microbiome correlated with higher white blood cell counts and hsCRP levels. Le Chatelier *et al.* reported that the metabolic phenotype of subjects with an LGC correlated with increased insulin resistance, higher levels of triglycerides and free fatty acids, decreased high density lipoprotein-cholesterol, as well as a marked inflammatory phenotype compared with individuals with high gene count of the gut microbiome. These and other data suggest that individuals with an LGC suffer from metabolic disturbances leading to an increased risk of diabetes mellitus, dyslipoproteinemia and pro-inflammatory status which might ultimately lead to ACVD (24,38,39).

The abundance of the genus *Bifidobacterium* was inversely related to levels of LPS and hsCRP. *Bifidobacterium* belongs to the Actinobacteria phyla, whose abundance is related to a healthier diet with an increased intake of whole grain cereals and certain vegetables (e.g. black-eyed peas) (40). Several *Bifidobacterium* taxa are used as probiotics, of which a recent meta-analysis revealed protective effects for cellular immune function (41). *Bifidobacterium* species are associated with gut barrier functions, a reduction in systemic inflammation and a reduction in the incidence of diabetes in mice (42).

The abundance of the genus *Faecalibacterium* and species *F. prausnitzii* were inversely correlated with hsCRP and IL-17. Besides showing negative correlations with inflammatory markers in individuals with obesity, the proportions

of *F. prausnitzii* were lower in patients with a history of stroke, in which the proportion also related with disease severity (43). *Faecalibacterium* is regarded as a next-generation probiotic for its several health-promoting and anti-inflammatory properties (44).

In general, lower levels of *Ruminococcaceae* and *Ruminococcus* are associated with higher levels of hsCRP and IL-6. However, opposite correlations have been observed, too; the genus *Ruminococcus* was highly positively correlated with both plasma TMA and TMAO levels, as well as atherosclerotic lesion area in female ApoE^{-/-} mice (45). Another large study seems to confirm this relation as they found that the abundance of *Ruminococcus gnavus* (*R. gnavus*) was higher in ACVD patients compared with controls (46). However, it is important to mention that *R. gnavus* species were reassigned to the genus *Blautia* in 2008 on the basis of 16S rRNA gene sequencing and therefore belong to the family *Lachnospiraceae* (47).

The abundance of *Prevotella* was inversely associated with LPS and hsCRP. Furthermore, individuals with obesity have a lower abundance of *Prevotella* species in their gut (48). However, large differences have been observed within this genus. Human studies have linked the increased abundance of *Prevotella* species at mucosal sites to localized and systemic disease, including rheumatoid arthritis, metabolic disorders, as well as low-grade systemic inflammation (49). *Prevotella* mediates the inflammatory response via toll-like receptor (TLR)2 activation and Th17 immune response. This *Prevotella*-mediated mucosal inflammation can lead to systemic inflammation. Certain *Prevotella* species are also suggested to play an important role in the pathophysiological relation between periodontitis and ACVD. Patients with ACVD have an enhanced abundance of *Prevotella nigrescens* (*P. nigrescens*) in subgingival plaques (50).

Gut microbiota in cardiovascular disease

The number of studies performed in humans to investigate the direct role of the gut microbiota in ACVD is relatively limited. Recently, the largest study in patients with ACVD was conducted by performing a metagenome-wide association study on stools from 218 individuals with ACVD and 187 healthy controls (46). The abundance of *E. coli*, *Klebsiella spp*, *Enterobacter aerogenes*, *R. gnavus*, *Eggerthella lenta*, *Streptococcus spp*, *L. salivarius*, *Solobacterium moorei* and *Atopobium parvulum* were elevated in patients with ACVD. In contrast, *Roseburia intestinalis*, *F. prausnitzii*, *Bacteroides spp*, *P. copri* and *Alistipes shahii* were relatively depleted in individuals with ACVD. Only smaller cohort studies and studies with methodological limitations have been published previously. In 2015, a study was published in which patients with large-artery atherosclerotic ischemic stroke and transient ischemic accident patients ($n = 141$) showed increased levels of Proteobacteria and reduced amounts of *Bacteroides*,

Prevotella and *Faecalibacterium* compared with 94 non-matched controls by 16S rRNA gene analyses (43). In another small cohort study, the genus *Collinsella* was found to be enriched, while the genera *Roseburia* and *Eubacterium* were depleted in 12 symptomatic atherosclerosis patients compared with 13 controls (51). The apparently beneficial effects of *Bacteroides*, *Faecalibacterium*, *Roseburia (intestinalis)* and *Alistipes spp* have also been found in our review, as they relate to lower levels of inflammatory markers. Next to this, higher levels of *E. coli*, *Klebsiella spp* and *Eggerthella lenta* associated with higher levels of inflammatory markers.

Quality and limitations of included studies

Gut microbiome in relation to white blood cell count

Several studies showed a possible association of the gut microbiome with blood cell counts. The first study by Mikelsaar *et al.* had a number of limitations (28), in particular, focusing only on specific strains of *Lactobacillus* species. The associations could furthermore have been influenced by the inclusion of subjects with osteoarthritis and the lack of adjustment in the analysis for smoking status. Also, a large number of individuals were taking probiotics, which may have influenced the microbiota composition. The study by Schirmer *et al.* included only healthy and mainly young individuals of which the cytokine responses to different stimuli may not be influenced by meta-inflammation (27). The association of gut microbiome with cell counts was also addressed in a large cross-sectional study by our research group (52), where eight different blood cell types were measured and related to microbiome composition, diversity and pathways. However, as we also included a small number of individuals with inflammatory bowel disease, this study was not included in our systemic review. This study identified 17 bacterial species that related to four different blood cell types and IL-6 levels. A limited number of species/genera were also found in the current review to be related to markers of chronic low-grade inflammation. For example, *Eubacterium ventriosum* correlated negatively to the number of leukocytes and granulocytes, while *R. torques* correlated positively to the number of lymphocytes. We summarized the relationship between bacterial species, blood cell counts, as well as IL-6 as discussed in this study in Table S5. After correcting for diet, medication, various physiological and biomedical measures, self-reported diseases and smoking status (in total > 200 intrinsic and environmental factors), no significant association of cell counts was observed with bacteria and pathways. This observation may reflect that the reported cell counts are not dependent on the gut microbiota; however, it is also likely that taking into account all available phenotypes to the analysis model leads to overcorrection, and therefore

missing the true-positive results or the association is too small to be detected within the given sample size.

Gut microbiome in relation to (hs)CRP

To measure low levels of hsCRP, a high sensitivity analysis needs to be used; however, not all studies adopted this method (30,34). The number of individuals used in the analysis also varied from 12 to 292. The largest study by Le Chatelier *et al.* (26) also used the most robust method to analyse the gut microbiome, quantitative metagenomics. This contrasts the study by Rajkumar *et al.* that cultured and enumerated only six different species (33), or the study by Brignardello *et al.* who used G + C peak content analysis and was unable to find a direct relation between specific gut microbial species and CRP levels (34).

Gut microbiome in relation to cytokines

In addition to the large differences in gut microbiome composition, differences in the cytokine measurements exist. The study by Schirmer *et al.* investigated cytokine responses *ex vivo* in PBMCs and whole blood stimulations with five different microbial pathogens (27), while the other studies measured circulating cytokines in blood.

Gut microbiome in relation to lipopolysaccharide

A major limitation of all the three studies investigating the relation to LPS is the small sample size, with a maximum of 49 participants in the study of Dao *et al.*, reducing the power to detect significant associations. LPS levels in the study by Radilla-Vazquez *et al.* and Clemente-Postigo *et al.* were assessed by the limulus amebocyte lysate assays; however, large differences were observed in the concentrations with a mean level of LPS of 0.16 EU/mL in Spanish cohort (with a mean age of 48 and mean body mass index (BMI) of 28) (23) and a mean LPS level of 1.14 EU/mL in the normal weight and 1.22 EU/mL in the young Mexican adults with obesity (36). It should be noted that measuring circulating LPS is notoriously difficult.

General limitations

After reviewing the literature, we could only include 14 studies that investigated the relationship between gut microbiota and markers of chronic low-grade inflammation in humans. Because the techniques used to analyse the gut microbiome were suboptimal in most studies, several of these studies could only investigate the gut microbiota at a specific taxonomic level. However, contradictory associations can exist in the relationship between different taxonomy levels with clinical markers. As already described before, we found an inverse correlation between the genus *Prevotella* and inflammatory markers, while increased abundance of certain *Prevotella* species was associated with low-grade inflammation in systemic diseases, such as rheumatoid arthritis (49). This emphasizes the complex

networks among bacterial groups and the large functional differences between species and strains, which should be kept in mind when interpreting the results. Larger studies with state-of-the-art gut microbiome analysis should further investigate this important association. Another important limitation is the heterogeneity between study populations. As mentioned before, the study by Schirmer *et al.* included only healthy and mainly young individuals of which the cytokine responses to different stimuli may not be influenced by meta-inflammation (27). This contrasts investigations by Claesson *et al.* where mainly older individuals were included (30).

A major limitation in all studies is the cross-sectional design, which cannot prove causal relationships. Different human intervention studies have been performed with probiotics (53) and even faeces transplantation (54). However, none of these studies investigated the specific effects of the microbiome species described here in relation to inflammatory markers. One meta-analysis demonstrated a protective role for several taxa of *Bifidobacterium* for cellular immune function in humans (41). Another limitation is the diversity in the statistical analysis methods. This depended mainly on the size of the study and the method of analysis of the microbiome. There is some hierarchy in the used statistical methods. The larger studies were able to perform regression analysis adjusting for covariates and multiple comparisons. Other studies used correlation analysis, either Pearson's or Spearman's without corrections. A number of studies only compared differences between subgroups of subjects using the Kruskal–Wallis, ANOVA or Mann–Whitney U test. In Table 1, we give an overview of the applied statistical methods.

Potential underlying mechanisms

Potential underlying mechanisms are summarized in Fig. 2 Our review focused on human studies, as humans and mice differ significantly in their gut microbiome composition and the pathophysiology of cardiovascular disease. Therefore, it remains to be determined in the future whether data gathered in mice can be directly extrapolated to humans. However, the human studies are mainly observational and therefore provide limited insight in the underlying mechanisms. Because the majority of mechanistic studies are performed using animal models, we included these data in this part of the discussion on potential underlying mechanisms (Table 3).

Pathogen-associated molecular patterns

A possible mechanism for the pro-inflammatory cytokines is the appearance of pathogen-associated molecular patterns (PAMPs) derived from microbiota in the gut and the circulation. Such PAMPs can be sensed by PRR. After activating a PRR, the inflammasome, an important part of our innate

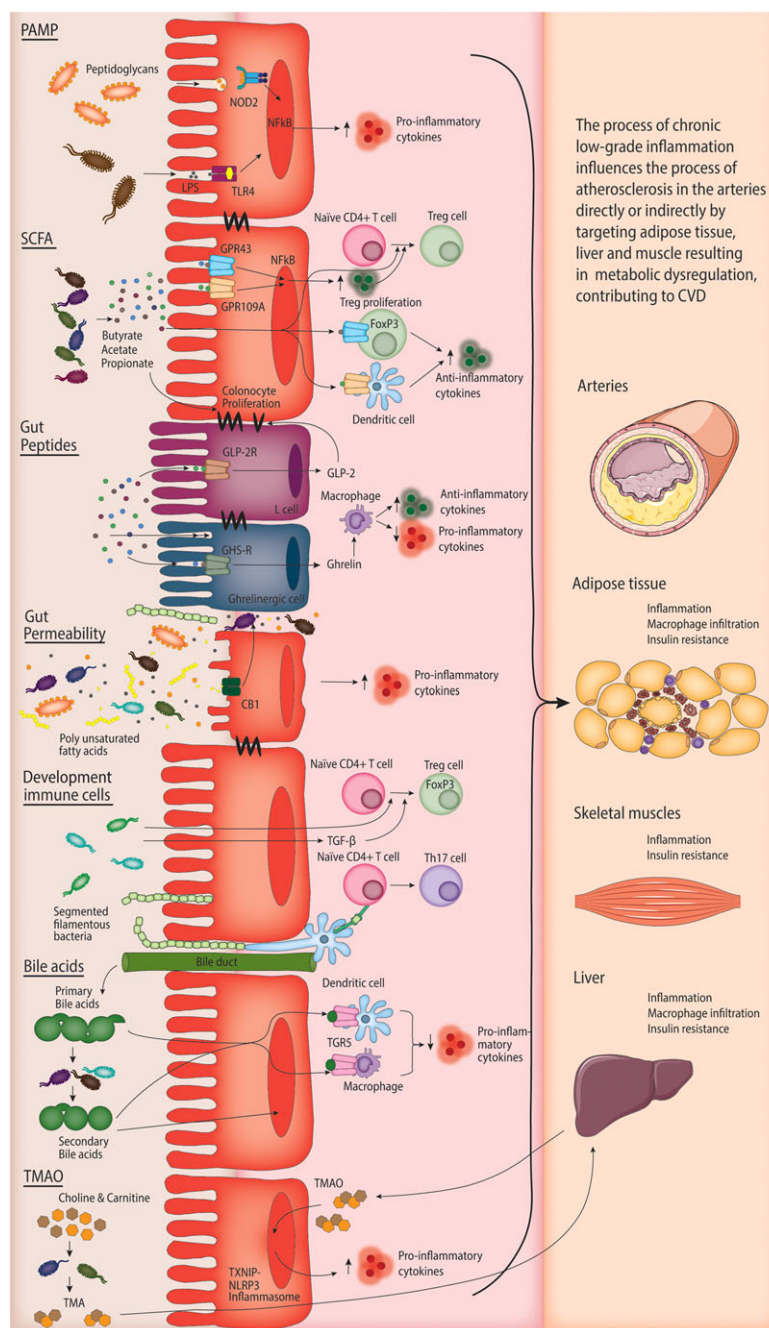


Figure 2 Summary of possible gut microbiota derived mechanisms able to influence the process of chronic low-grade inflammation in ACVD. PAMPs, like peptidoglycans and LPS can stimulate NOD2 or TLR4, respectively, and stimulate the production of pro-inflammatory cytokines via NF κ B activation as part of the inflammasome. SCFAs can stimulate colonocyte proliferation, but also GPR43 and GPR109A activation which leads to the induction of Treg cells and the production of anti-inflammatory cytokines by Treg and dendritic cells. SCFAs also stimulate the production of gut peptides. GLP-2 production improves tight junctions and mucosal barrier function. Specific species have an influence on these gut permeability mechanisms. Polyunsaturated fatty acids can stimulate the CB1 receptor, which promotes gut permeability. Specific species have shown to enhance Treg cell abundance and induce anti-inflammatory molecules. On the other hand, segmented filamentous bacteria can activate TGF- β and thereby promote the development of Th17 cells. Primary and secondary bile acids can inhibit NF κ B-dependent transcription of pro-inflammatory cytokines from macrophages and dendritic cells via the TGR5 receptor (55). Taurine increases the production of anti-inflammatory cytokines via NLRP6 inflammasome. TMAO has shown to activate TXNIP-NLRP3 inflammasome and increase the production of pro-inflammatory cytokines. PAMP *pathogen associated molecular pattern*; SCFA *short chain fatty acid*; NOD2 *nucleotide-binding oligomerization domain-containing protein 2*; NF κ B *nuclear factor kappa B*; LPS *lipopolysaccharide*; TLR4 *toll-like receptor 4*; GPR *G protein-coupled receptor*; Treg *regulatory T cell*; GLP-2R *glucagon like protein-2 receptor*; GLP-2 *glucagon like peptide-2*; GHS-R *Growth hormone secretagogue receptor*; CB1 *cannabinoid receptor 1*; TGF- β *transforming growth factor beta*; TGR5 *Takeda G-protein-coupled receptor 5*. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 3 Summary of findings and possible mechanisms

	Inflammatory marker					Possible mechanism	
	LPS	WBC	hsCRP	Cytokines IL-6 IL-8 IL-17			
Bacterial diversity and taxonomy							
↓ Alpha diversity or gene count		↑	↑				Reduced access to complex carbohydrates with less production of SCFA
↓ genus <i>Bifidobacterium</i>	↑		↑				Gut barrier protection
↓ genus <i>Faecalibacterium</i> (and species <i>F. prausnitzii</i>)			↑			↑	Production SCFA
↓ genus <i>Ruminococcus</i> (and family <i>Ruminococcaceae</i>)			↑	↑			Production SCFA
↓ genus <i>Prevotella</i>	↑		↑				PAMPs

immune system that responds to danger signals (56), is activated with an enhanced production of the transcription factor nuclear factor kappa B (NF-κB). Different bacterial components have been demonstrated to be a PAMP and thereby initiate the inflammasome pathway with NF-κB production. LPS is believed to confer its deleterious effect on the cardiovascular system mainly via upregulation of pro-inflammatory cytokines, a process that is mediated via TLR4. Importantly, LPS derived from different gut microbial species are not equally toxigenic and can differently induce TLR4 signaling (57). LPS forms a complex with LPS-binding protein which then binds to CD14 (mainly from macrophages, but also neutrophils, monocytes and hepatocytes) or nucleotide-binding oligomerization domain (NOD)1, to initiate an acute immune response mainly via TLRs and NF-κB (58). Humans with obesity exhibit elevated levels of LPS-binding protein (59), possibly contributing to the explanation of increased LPS levels in these individuals. Endotoxins derived from gut bacteria are normally detoxified in the liver. However, when the influx increases, it may exceed the capacity of Kupffer cells and thereby enter the systemic circulation (60). The importance of LPS in ACVD has been demonstrated for the first time by the Bruneck study (61). Subjects with levels exceeding 50 pg mL⁻¹ faced a threefold higher risk of developing incident atherosclerosis. Subclinical endotoxemia was shown in mice to accelerate atherosclerosis by programming monocytes into a non-resolving inflammatory state (62). High fat diet (HFD)-fed mice treated with antibiotics showed an increase in *Prevotella* species and a decrease in endotoxemia (63). This observation is in accordance with the findings by Clemente *et al.* and Claesson *et al.*, which describe an inverse relationship between *Prevotella* species, LPS and IL-6 levels (23,30). However, no causal relationships has been shown between the abundance of *Prevotella* species and levels of inflammatory markers. Another important PAMP is the peptidoglycan that can activate the NOD receptors. NOD receptors can recognize bacterial determinants once they are phagocytosed by macrophages or dendritic cells. HFD-fed mice deficient in NOD2 show increased bacterial translocation and insulin resistance (64). A role for NOD2 in atherosclerosis has also been

demonstrated in human genetic and mouse knockout studies (65,66).

Short chain fatty acids (SCFAs)

Short chain fatty acids are produced by the microbiome through degradation of dietary fibres; the most important SCFAs are butyrate, acetate and propionate (67). An important function of these SCFA is to promote colonocyte proliferation, which can improve insulin resistance in mice via promotion of energy expenditure and induction of mitochondria function (68,69). SCFAs are also important in the regulation of appetite via gut hormones (67). Activation of different G protein coupled receptors (GPR), mainly GPR41 and GPR43, has been shown to suppress inflammation, partly via epithelial survival and integrity, but also via induction of regulatory T (Treg) cells. Activation of GPR109A by butyrate has been shown to affect colonic macrophages and dendritic cell maturation and function, which stimulates the induction of transforming growth factor (TGF)-β to promote the induction of Treg cells and IL-10 producing T cells (70). Besides this, butyrate and acetate act as histone deacetylase inhibitors in dendritic cells and T cells with modulation of gene expression and contributing to epigenetic modulation (71). This induces extrathymic generation of Treg cells from naïve T cells and limits the secretion of pro-inflammatory cytokines (72). *F. prausnitzii*, *Eubacterium rectale*, *Eubacterium hallii* and *R. bromii* appear to be responsible for the majority of butyrate production (73); this makes sense given the inverse correlation of these species with hsCRP and some pro-inflammatory cytokines (27,30–32,35).

Gut peptides

Different gut hormones have been shown to support epithelial barrier formation and anti-inflammatory properties. Ghrelin levels are decreased in response to HFD and can promote lymphocyte development in the primary lymphoid organs (74). Cani *et al.* reported that higher endogenous glucagon-like peptide 2 (GLP-2) production is associated with an improvement of the mucosal barrier function, improved tight junctions, as well as decreased plasma LPS concentrations. This was associated with an increased

abundance of *Bifidobacterium* species (75) and might be one of the reasons for the inverse observation between *Bifidobacterium* species and inflammatory markers (23,33). Obesity can also enhance the secretion of the pro-inflammatory serotonin. Indigenous spore-forming bacteria, mainly *Clostridium* species, can promote serotonin production from colonic enterochromaffin cells (76). In mouse models, it was shown that serotonin could recruit T cells and stimulate the production of pro-inflammatory cytokines (77,78).

Gut permeability and endocannabinoid system

High fat diet negatively influences gut barrier function by inducing a number of changes in the gut microbiome and the endocannabinoid (eCB) system. The eCBs are derivatives of polyunsaturated fatty acids that can activate the G-coupled receptors CB1 and CB2. When the CB1-receptor is activated, an increase in the intestinal epithelial barrier permeability is induced, which can contribute to circulating LPS concentrations. HFD increases the expression of the colonic CB1 receptor of the eCB system (79). Treatment with prebiotics leading to a change in gut microbiome was associated with a reduction in metabolic endotoxemia and improvement of gut barrier function via reduction of the CB1 receptor (80). Activation of CB2 receptor has been shown to improve glucose tolerance in rats (81). CB2 receptor expression can mainly be increased by *Lactobacillus* supplement, specifically *L. acidophilus* and decreased by *Clostridium* supplement (81,82). Administration of *A. muciniphila* in mice increased the levels of eCBs in the small intestine, which via activation of the CB2 receptor contribute to the anti-inflammatory effects and improved gut barrier function (83). Besides its effects on gut permeability, the eCB system has also been shown to control food intake and energy expenditure both centrally and peripherally (84). Furthermore, the microbiome can influence gut barrier function and inflammation directly via specific species like *Bifidobacterium* species as well as via the production of SCFA (75,85,86). However, it should be noted that even if relations exist between the composition of the gut microbiota and gut permeability, the direct involvement of specific gut microbes and/or metabolites needs further elucidation.

Bile acids

The gut microbiota can also influence the inflammatory state of the host through an extensive involvement in bile acid homeostasis (87). Primary bile acids are derived from the oxidation of cholesterol in the liver and are secreted into the intestine to solubilize lipids for absorption. Microbial bile salt hydrolase, mainly produced by gut bacteria from the genera *Lactobacillus*, *Bifidobacterium*, *Clostridium* and *Bacteroides*, can deconjugate primary bile acids to secondary bile acids (87). Free taurine, an amino acid to conjugate bile acids, can enhance the activation of the NOD-like

receptor family pyrin domain containing 6 (NLRP6) inflammasome and thereby increase production of IL-18 by the intestinal epithelium, which supports epithelial barrier function (88). Primary and secondary bile acids can inhibit NF- κ B-dependent transcription of pro-inflammatory cytokines from monocytes, macrophages, dendritic cells and kupffer cells via the farnesoid X and Takeda G-protein-coupled receptor (TGR)5 receptor (55).

Development/differentiation of innate immune cells

An important role for the gut microbiome exists in the development/differentiation of immune cells. In germ-free mice fed a diet with low LPS content, the mesenteric lymph nodes and Peyer's patches contained fewer CD4⁺ T lymphocytes, mainly due to fewer amounts of CD4⁺ FoxP3⁺ Treg lymphocytes (89). Species belonging to the *Clostridium* cluster IV can enhance Treg cell abundance and induce anti-inflammatory molecules (90). This is in accordance with the seemingly protective role of *Faecalibacterium* and *Ruminococcus* species (30,31). Furthermore, intestinal Th17 cell development is dependent on the microbiome. Dendritic cells and macrophages in the lamina propria sense segmented filamentous bacteria, such as Clostridia related bacteria, and produce IL-6 and integrin molecules that contribute to the activation of TGF- β and thereby promote Th17 cell development in the intestine (91,92). It has been shown that jejunal T cell inflammation in human obesity correlates with decreased enterocyte insulin signaling (93). High fat feeding has also been shown to influence the immune cell populations in the digestive tract. In mice, after 12 weeks of HFD, the proportion of Th1 cells and CD8⁺ T cells increased in the small bowel and colon, while the proportion of Treg cells decreased (94).

Trimethylamine-N-oxide (TMAO)

The most convincing link between the gut microbiota and ACVD is trimethylamine-N-oxide (TMAO) (95), a metabolite derived from dietary choline or carnitine through the action of gut bacteria. Bacteria from the phylum Proteobacteria and Firmicutes (96). TMA is converted in the liver to TMAO via flavin monooxygenase 3 (FMO3) (97). TMAO has shown to promote recruitment of activated leukocytes to human endothelial cells (98). This was supported by another *in vitro* study in which TMAO activated TXNIP-NLRP3 inflammasome and IL-1 β and IL-18 were released in a dose- and time-dependent manner, while endothelial nitric oxide synthase (eNOS) and production of nitric oxide (NO) were inhibited (99). The same research group later found that TMAO up-regulated vascular cell adhesion molecule-1 (VCAM-1) expression and promoted monocyte adherence (100). *LDLR*-deficient mice fed a choline diet showed elevated inflammatory gene expression compared with controls (98). *ApoE*-deficient mice treated with antibiotics had lower TMAO levels and a reduced

atheroma size, while supplementing the diet with 1% choline increased foam cell formation, which could be prevented by antibiotics (101). Faecal transplantation from high TMAO-producing mice to *ApoE*-deficient mice accelerates atherosclerosis (102). Plasma TMAO levels are an independent prognostic marker for ACVD in patients undergoing elective coronary angiography (95).

As discussed above, the gut microbiome can influence low-grade inflammation via several mechanisms. Because low-grade inflammation is a hallmark of obesity that is linked to ACVD, the gut microbiota composition could influence the risk for atherosclerosis and harbour possible diagnostic and therapeutic options. One human study demonstrated a positive result for faecal transplantation with regard to obesity-induced insulin resistance (54). Eighteen insulin resistant men who were randomized to duodenal infusion of microbiota from either a heterologous lean donor or an autologous faecal microbiota showed improvement in insulin sensitivity 6 weeks after infusion of microbiota from lean donors. The improvement in insulin sensitivity in recipient patients correlated with an increase in the number of butyrate-producing bacteria, pointing towards a regulatory role for butyrate derived from gut microbial metabolism leading to improved insulin sensitivity. Multiple studies are currently being undertaken to investigate the possibilities of faecal transplantation as treatment option for obesity and obesity-related complications. Indirect evidence for the influence of the gut microbiota on ACVD comes from animal studies. *ApoE*-deficient mice reared under germ-free conditions fed a low cholesterol diet exhibited atherosclerotic plaques in the aorta, in contrast to conventionally reared *ApoE*-deficient mice fed a low cholesterol diet who did not develop atherosclerotic aortic plaques (103). Germ-free mice receiving a faecal transfer from genetically or diet-induced obesity mice with obesity developed greater adiposity on an HFD than did recipients of microbiota from lean mice (18,104,105). Similarly, faecal transfer from humans into germ-free mice mirrored the adiposity of its donor (106). Not only the adiposity status, but also its complications, like insulin resistance and non-alcoholic fatty liver disease, were demonstrated to be transmissible via the gut microbiota (104,107). This has been shown to be driven (at least partly) by pro-inflammatory microbial products (63,108).

Perspective

While the gut microbiota is increasingly recognized as a determinant of obesity, its influence on the development of low-grade inflammation and ACVD remains largely unexplored. Future studies should investigate the underlying pathophysiology, with a focus on mechanisms leading to low-grade inflammation. This is especially important because the CANTOS trial has already shown that inhibiting

a pro-inflammatory pathway reduced ACVD related mortality (10). The gut microbiota could serve as a diagnostic marker by which a more pro-inflammatory state could be detected in an early stage and could predict the risk to develop certain ACVD states. For these diagnostic options, first the gut microbiota leading to low-grade inflammation in humans needs to be established in more detail. Well-designed clinical studies with state-of-the-art analysis of the gut microbiota, like shot gun metagenomics, are required. By using metagenomics analysis, not only the taxonomy of the gut microbiota, but also the functionality can be investigated and can possibly contribute to new diagnostic strategies. Future studies should include subjects at risk for ACVD, such as individuals affected by obesity. Future studies should also focus on prospective and intervention studies. Potentially, the use of prebiotics or probiotic strains or faecal microbiota transplantation via capsules could become a promising therapeutic option to prevent low-grade inflammation and ACVD in the future.

Conclusion

In this review, we have provided an overview of the relationships between the gut microbiota and markers of low-grade inflammation in humans and discussed the possible mechanisms. These data reinforce the importance of human research into the gut microbiota in relation to the innate and adaptive immune system to prevent and treat ACVD.

Conflict of interest statement

No conflict of interest was declared.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article. <https://doi.org/10.1111/obr.12750>

Table S1. Extended version of characteristics 15 of studies

Table S2. Quality assessment of observational 20 studies

Table S3. Quality assessment of non-randomized intervention 24 studies

Table S4. Quality assessment for randomized Intervention 28 studies

Table S5. Findings from large cross-sectional study by 35 Zhernakova *et al.*

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